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Evolution of sex-biased gene expression in a dioecious plant

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Abstract

Separate sexes and sex-biased gene expression have repeatedly evolved in animals and plants, but the underlying changes in gene expression remain unknown. Here we studied a pair of plant species, one in which separate sexes and sex chromosomes evolved recently and one which maintained hermaphrodite flowers resembling the ancestral state, to reconstruct expression changes associated with the evolution of dioecy. We find that sex-biased gene expression has evolved in autosomal and sex-linked genes in the dioecious species. Most expression changes relative to hermaphrodite flowers occurred in females rather than males, with higher and lower expression in females leading to female-biased and male-biased expression, respectively. Expression changes were commoner in genes located on the sex chromosomes than the autosomes and led to feminisation of the X and masculinisation of the Y chromosome. Our results support a scenario in which sex-biased gene expression evolved during the evolution of dioecy to resolve intralocus sexual conflicts over the allocation of resources.

Females and males of many plant and animal species differ in morphological, physiological and ecological characteristics, despite their overall genetic similarity [1,2]. Such sexual dimorphism can evolve through sex-limited or sex-biased expression of genes that are present in both sexes, or through complete sex-linkage, when a gene or allele is restricted to the genome of just one sex [1-5]. Sex-biased and sex-limited gene expression, and enrichment of such genes in fully sex-linked regions, are well documented in animals [1,6-8], including humans [9], and have recently been studied also in plants and algae [10-13], but the evolutionary changes that actually led to expression differences between the sexes remain unknown.

Sex-biased gene expression can evolve through changes in expression in either one or both sexes. For example, female-biased expression may evolve by increased expression in females, decreased expression in males, or a combination of both (Fig. 1a, scenarios I-III). In many dioecious organisms, these evolutionary changes cannot be studied because separate sexes evolved too long ago. Species in which they evolved more recently, such as some plants, are therefore of great interest, because gene expression changes can be inferred from comparisons with related species without separate sexes, which should often represent the ancestral state (Fig. 1b). Such changes provide new information about the role of sex-linked and sexually antagonistic genes in the evolution of separate sexes [14].

Here, we study the evolution of sex-biased gene expression in dioecious *Silene latifolia* Poiret (White Campion), a plant model for sex

chromosome evolution [15-20]. In the genus *Silene*, gynodioecy, the co-existence of hermaphrodites and females in the same population, represents the ancestral state, and dioecy (separate sexes) has evolved at least twice independently [21]. *Silene vulgaris* (Moench) Garcke is gynodioecious and closely related to *S. latifolia* [15], in which female and male flowers and inflorescences are sexually dimorphic [22] (Fig. 2a) and both fully and partially sex-linked quantitative trait loci affecting sexually dimorphic traits have been inferred [23]. *Silene latifolia* has an XY sex-determination system with heteromorphic sex chromosomes that have evolved within the past 5-10 MY [24,25]. Its Y chromosome is much less degenerated than in ancient animal sex chromosome systems [17,18,26].

We used comparative mRNA-seq transcriptome sequencing to assess gene expression differences between *S. latifolia* females and males and investigate evolutionary changes in gene expression in each sex from the likely ancestral state represented by flowers of *S. vulgaris* hermaphrodites. We further test for differential representation of sex-biased genes on the sex chromosomes and autosomes, and assess allele-specific changes in the expression of sex-linked genes to test for feminisation and masculinisation of the X and Y chromosome, respectively. We find that the evolution of sex-biased expression in *S. latifolia* primarily involved changes in females, and that the different selective forces acting on the sex chromosomes have not yet led to accumulation of genes with female-biased and male-biased expression on the X and Y chromosome, respectively, but to subtler expression

changes causing feminisation of the X and masculinisation of the Y chromosome.

Results

The extent of sex-biased gene expression.

We analysed mRNA-seq data from Illumina 100 bp paired-end reads obtained from developing flower buds and rosette leaves of female and male *S. latifolia*, and flower buds of hermaphrodite *S. vulgaris*. We obtained 145 Gb of RNA-seq from flower buds of seven female and seven male *S. latifolia* individuals, and 41 Gb from rosette leaves from four individuals of each sex. From the flower buds of five *S. vulgaris* hermaphrodites we obtained 33 Gb of transcriptome data. 58% and 57% of the *S. latifolia* reads from flower buds and rosette leaves, respectively, and 44% of the *S. vulgaris* reads, matched sequences in the *S. latifolia* flower bud reference transcriptome (for details see Supplementary Table 1). The lower percentage for *S. vulgaris* probably reflects sequence divergence between the two species [24].

We used flower buds in our expression analyses because sexual dimorphism in *S. latifolia* is stronger for flower and inflorescence traits than for other characters [22]. However, some sex differences in gene expression in buds must be due simply to the presence or absence of the sex organs (referred to as “primary differences“ in Fig. 1b). Therefore, before quantifying sex-bias in gene expression, or counting numbers of genes with evolved expression differences between the sexes, we

excluded all 903 contigs exhibiting sex-limited expression in this data set (i.e. expressed in buds of only one sex in *S. latifolia*,). Among the remaining 11,366 *S. latifolia* contigs with at least some expression in buds of both sexes, many showed significant sex differences in expression (Supplementary Fig. 1, Supplementary Table 2). The results are robust to different normalisation procedures and estimators of gene expression differences (Supplementary Fig. 2), and, for 16 genes tested, agree well with qRT-PCR results (Supplementary Fig. 3; Spearman correlation; ϱ=0.92; p<0.0001). GO analysis revealed several biological processes that are significantly over-represented among female-biased genes but under-represented among male-biased genes, or vice versa, suggesting that sex-biased expression has evolved to support contrasting biological functions in *S. latifolia* females and males. Specifically, female-biased contigs are enriched for transcription factors involved in cell-cycle and developmental functions, but depleted in genes involved in catabolism (Supplementary Table 3), while male-biased contigs are enriched in genes involved in carbohydrate, lipid, and secondary metabolite metabolism, transport, and responses to various stimuli, and depleted in genes involved in nucleic acid metabolism and protein synthesis and modification.

We divided the contigs expressed in buds into autosomal, sex-linked (defined as contigs having both X- and Y-linked alleles) and X hemizygous contigs (sex-linked, but with an expressed copy on the X only). These categories were inferred from SNPs segregating in a full-sib family, using a probabilistic model [27]. 2,142 *S. latifolia* bud-expressed

131 autosomal contigs (16.8% of such contigs) had significantly sex-biased
132 expression (Supplementary Table 2), 7.2% with female and 9.6% with
133 male-biased expression (Fig. 2b). Sex-biased expression is commoner
134 among the 936 fully sex-linked contigs (28.8% overall, with respectively
135 13.8% and 15.0% having female and male-biased expression; Fig. 2b
136 and Supplementary Table 2). Female-biased expression of sex-linked
137 genes may reflect either higher expression in females or lower expression
138 in males caused by reduced expression of the Y-linked allele if dosage
139 compensation is absent or incomplete. These alternatives are notoriously
140 difficult to distinguish [28]. Incomplete dosage compensation is widely
141 observed in animals [1]. In *S. latifolia*, evidence for dosage compensation
142 has been reported [20,29], but not all genes are fully compensated [16,17,26].
143 Apparent female-biased expression due to incomplete dosage
144 compensation should be displayed in both flower buds and vegetative
145 tissues [8]. In order to exclude such genes, we therefore examined sex-
146 biased expression also in rosette leaves (which show overall less sex-
147 biased expression, see below). Of the 86 sex-linked contigs with female-
148 biased expression in flower buds that were sufficiently expressed in
149 rosette leaves, only 16 (18.6%) had female-biased expression
150 (Supplementary Fig. 4). Excluding these from our analysis, the sex
151 chromosomes still have a highly significant overrepresentation of contigs
152 with sex-biased expression (Fisher's exact test, $p < 0.0001$), but no
153 longer of contigs with female-biased expression (Fisher's exact test,
154 $p=0.7303$). The apparent over-representation of female-biased genes on

155 the sex chromosomes therefore probably reflects incomplete dosage
156 compensation in *S. latifolia*.

157 Many fewer genes showed sex-biased expression in rosette leaves than
158 in flower buds (Fisher's exact test, $p < 0.0001$), consistent with sexual
159 dimorphism in *S. latifolia* being stronger for flower and inflorescence traits
160 [22]. This difference was observed for both autosomal and sex-linked
161 contigs (18.7-fold and 3.84-fold lower, respectively, see Fig. 2b and
162 Supplementary Table 2). Similar findings in *Rumex hastatulus* [30] suggest
163 that sex bias may generally be low in plant vegetative tissues. As in buds,
164 genes with sex-biased expression in rosette leaves were over-
165 represented on the sex chromosomes (Fig. 2b). Among contigs
166 expressed in rosette leaves, female-bias was commonest, with 0.6% of
167 autosomal and 4.1% of sex-linked contigs showing female bias, and male
168 bias being significantly rarer (0.3% of autosomal and 3.4% of sex-linked
169 contigs, Supplementary Table 2, Fisher's exact test, $p < 0.0001$). The
170 higher frequency of female-biased contigs in leaves contrasts with our
171 finding of a higher proportion of contigs with male-biased expression in
172 buds. In *Asparagus officinalis* flower buds, genes with higher male than
173 female expression also predominated [11], potentially reflecting sexual
174 selection acting on floral and inflorescence traits [31,32].

175 **Evolution of sex-biased gene expression**

176 To investigate the evolutionary changes that have led to the observed
177 sex-biased gene expression in *S. latifolia*, we also estimated gene
178 expression in hermaphrodite flowers of gynodioecious *S. vulgaris*.

Because few genes show sex-biased expression in vegetative tissues of *S. latifolia* we focus on expression changes in buds. Expression levels of genes with no sex bias in expression in *S. latifolia* (white bars in Fig. 3) are largely unchanged in females and males, relative to *S. vulgaris* hermaphrodite flower buds, indicating that much of the gene expression changes between the two species relates to the evolution of separate sexes. Our results reveal fundamental changes in the transcriptome associated with the evolution of dioecy, resulting in both male- and female biases in expression (Fig. 1b), and revealing the changes that led to them. For both autosomal and sex-linked contigs in *S. latifolia* (Fig. 3), we found that the evolution of sex-biased expression mainly involves changes in females: female-biased expression (red bars in Fig. 3) is due primarily to higher expression in *S. latifolia* female buds, and the many genes with male-biased expression (blue bars in Fig. 3) primarily result from lower expression in females than in *S. vulgaris* hermaphrodites, implying many changes in autosomal and X-linked genes. For both autosomal and sex-linked contigs, gene expression changes in males are much smaller than those in females, although the variances are high for the more limited number of sex-linked contigs (Fig. 3). Similar patterns were also found for both X-hemizygous contigs and contigs whose genomic locations are unknown (called “undefined contigs” in Supplementary Fig. 5).

For most contigs whose expression was studied, we inferred whether they are autosomal or sex-linked, which required expression in both sexes, and therefore genes with primary expression changes due simply

204 to loss of sex organs and functions (see above) are not included.
205 Secondary gene expression changes, including up- or down-regulation of
206 genes following establishment of a unisexual type in a population (Fig.
207 1b), are of greater interest, and may often benefit the sex in which
208 expression is changed [33]. Assuming that expression changes affect
209 fitness and are under selection, rather than reflecting neutral divergence
210 (evidence for selection is described below), three evolutionary scenarios
211 are possible (Fig. 1a). When an expression change is advantageous only
212 for one sex, increased expression of a gene may occur specifically in that
213 sex, with the other sex retaining the ancestral expression level. For
214 example, if the ancestral expression state is optimal for males but
215 suboptimal for females, a female-specific increase will be advantageous,
216 whereas selection favours retaining the ancestral expression state in
217 males, as it is already optimal (Scenario I). Similarly, in scenario II, the
218 ancestral expression state exceeds the optimum for males, favouring a
219 male-specific reduction in expression. In scenario III, the expression level
220 of a gene in the ancestral hermaphrodite (before dioecy evolved) was
221 non-optimal for both sexes, potentially because of trade-offs [34], and this
222 was adjusted by evolutionary changes in both sexes after dioecy evolved.
223 Large expression changes in opposite directions in both sexes suggest
224 the evolution of changes in response to sexual antagonism at such genes,
225 increasing their expression in the sex where high expression is
226 advantageous, and reducing it in the other sex (Fig. 1a; scenario III).
227 Patterns corresponding to scenarios I and II are also compatible with the
228 hypothesis that sexual antagonisms underlie evolutionary changes in

gene expression between the sexes. If the optimal expression for one sex is the same as the ancestral state, sex-biased gene expression can evolve during the evolution of unisexuality, when expression is optimisd in the other sex to resolve ancestral antagonistic effects.

We inferred the directions of changes in expression by comparing the expression of female- and male-biased genes in dioecious *S. latifolia* with expression levels in hermaphroditic flowers of *S. vulgaris* (Supplementary Fig. 6). Approximately half of the genes with sex-biased expression could be assigned to scenarios I to III (Fig. 1a). Other changes leading to sex-biased expression are possible, such as increased (or decreased) expression in both sexes, relative to hermaphrodite flowers, but to different extents; these, however, cannot be distinguished from species-specific changes in expression between *S. latifolia* and *S. vulgaris* and are therefore not discussed. Of the autosomal contigs with male-biased expression, only a small proportion (14.9%) evolved through increased expression in males (Fig. 4a, I, blue bar), whereas 39.4% have undergone reduced expression in females (Fig. 4a, II, blue bar). In marked contrast, a large percentage (42.1%) of autosomal genes with female-biased expression in *S. latifolia* are more strongly expressed in *S. latifolia* females than in hermaphroditic *S. vulgaris* flowers (Fig. 4a, I, red bar), while only 11.2% evolved lower expression in males (Fig. 4a, II, red bar). The results are similar for the smaller number of sex-linked (Fig. 4a) and X-hemizygous contigs and contigs with undefined locations (Supplementary Fig. 7). Thus sex-biased expression in the dioecious *S. latifolia* evolved primarily through expression changes in females, rather

than males: higher expression in females led to female-biased expression, of many genes, whereas male biases evolved mainly through reduced expression in females.

The role of selection in the evolution of sex-biased gene expression

We estimated the proportions of genes with evidence for directional selection underlying gene expression changes under scenarios I and II above by computing ΔX values [35,36]. Our results indicate that the great majority of expression changes in females, but not in males, have been driven by selection. Fig. 4 a-b shows the fractions of genes in the top 25% of ΔX values whose directions of change correspond to scenarios I and II. We estimate that only about 50% of these autosomal expression changes in males can be attributed to directional selection, whereas our estimates are much higher for expression changes in females (73% for up-regulations creating female-biased contigs, and 96% for down-regulations creating male-biased contigs). Expression changes in one sex, without change in the other, were much commoner than significant expression changes in opposite directions in the two sexes (scenario III in Fig. 1a); almost all of these genes are male-biased in *S. latifolia* (Fig. 4c). In animals, male-biased genes are also often commoner than female-biased genes, and tend to evolve more rapidly, possibly because sexually antagonistic selection is stronger in males [1]. Consistently, male-biased expression in *Drosophila* was inferred to result primarily from adaptive changes in the male transcriptome [37]. In *S. latifolia*, although there are many male-biased genes, these do not indicate stronger sexually

antagonistic selection in males, because they evolved through reduced expression in females.

Sex-biased expression on sex chromosomes

In dioecious species, tertiary changes in gene expression may follow the evolution of sex chromosomes with non-recombining regions (Fig. 1b, Step iii) and include expression changes that are specific to the X and Y chromosome. Overall, the expression changes inferred for sex-linked contigs are consistent with those for autosomal contigs, but the proportion of genes with changes in males was slightly higher than for autosomal contigs (Fig. 4). Evidence for a selective advantage of expression changes on the sex chromosomes was again strongest for changes in females (82% and 100% of contigs with higher and lower expression in females, respectively, are in the top 25% of ΔX values; Fig. 4b), and higher proportions of contigs were inferred to have decreased expression as a consequence of selection than to have undergone increases (Fig. 4b), suggesting that selection may be strong to reduce fitness costs at loci on the sex chromosomes that have fixed sexually antagonistic alleles.

In animals such as *Drosophila* [7] and mice [6] with evolutionarily much older sex chromosomes, most functional Y-linked genes have been lost as a consequence of Y chromosome degeneration, rendering the majority of X-linked genes hemizygous in males. Dosage compensation systems have evolved in these species, compensating for low expression from degenerated Y-linked genes and X chromosomes have evolved an overrepresentation of genes with female-biased expression [38]. Such a

feminisation of the X is predicted by theory for hemizygous loci (reviewed in [1]) whereas the Y chromosomes are enriched for genes with male-biased expression among their few remaining genes (masculinization) [39-42]. In *S. latifolia*, up to 45% of Y-linked genes are not expressed [20]. Nevertheless, Y chromosome degeneration is much less extensive than in old animal sex chromosomes, and X-hemizygous genes studied are apparently not dosage compensated [16,17,26]. Together, these differences prevent direct comparison with the much older animal sex chromosomes.

To assess whether subtler gene expression changes have evolved on the *S. latifolia* X and Y chromosome, we examined sex-linked genes with copies expressed from both the X and Y chromosome. Expression ratios of these genes (per X-linked allele, see Supplementary Methods) revealed that genes with equal expression in both sexes express their X-linked alleles with equal intensity in females and males (Fig. 5a). However, the expression per X-linked allele is significantly higher for contigs with female-biased expression, and lower when the gene is male-biased (Fig. 5a; Wilcoxon-test, $p < 0.0001$ for both comparisons), suggesting subtle feminisation and de-masculinisation of the *S. latifolia* X chromosome.

To assess whether the Y chromosome has similarly evolved a degree of masculinisation, a simple comparison of expression ratios of Y-linked versus X-linked alleles may be inappropriate if dosage compensation in *S. latifolia* [20,29] is achieved through increased X/Y expression in males. Instead, therefore, we compared the expression of Y-linked alleles in *S. latifolia* with that of the homologous genes in hermaphrodite *S. vulgaris*

(not sex-linked). Compared with contigs without sex bias in expression, contigs with male-biased expression in *S. latifolia* indeed had higher Y/*S. vulgaris* allele expression ratios (Y/0.5*AA in Fig. 5b; Wilcoxon-test, $p < 0.01$), and Y-linked alleles of contigs with female-biased expression had lower ratios (Wilcoxon-test, $p < 0.001$). Consistent patterns were seen for Y/X expression ratios in males (Supplementary Fig. 8; the ratios are correlated; Spearman correlation, $\rho = 0.598$, $p < 0.001$; Supplementary Fig. 9). The weakly negative median expression ratio of contigs with unbiased expression is in agreement with other studies evidencing Y chromosome degeneration in *S. latifolia* [17,18,20,26,29,43,44] and the higher ratio of contigs with male-biased than female-biased expression supports a scenario in which degeneration of Y-linked genes is retarded by haploid selection acting on pollen-expressed genes [17]. We conclude that the *S. latifolia* Y chromosome has undergone some masculinisation and thus contributes to male-biased expression of sex-linked genes.

Discussion

Our finding that sex-biased gene expression in a dioecious plant has most often evolved through decreased transcription, predominantly in females, is consistent with the occurrence of sexual antagonism in the hermaphrodite ancestor, specifically with intralocus sexual conflicts in which high expression levels of many genes benefitting male functions in the hermaphrodite, are detrimental in females [33]. Similarly, the smaller, but still substantial, number of genes that underwent expression changes only in males suggests that males benefit from changed expression of

some genes that experienced trade-offs in the hermaphrodite, but were fixed for female beneficial/male detrimental alleles [34]. Together, our results suggest that conflict over gene expression in hermaphrodite flowers of *S. vulgaris* led to an outcome closer to the optimum for male than female functions, and that sex-biased gene expression may have been involved in re-allocating resources during the evolution of dioecy in *S. latifolia*, resolving such conflicts. Such resource reallocation was apparently more important for females than for males, compatible with female plants often being resource limited [14,45], and with Darwin's [46] idea that resource compensation is a major factor in the evolution of dioecy.

While our results support the hypothesis that sex-biased expression has evolved to reduce intralocus sexual conflict, it remains unknown what fraction of genes with sex-biased expression evolved through conflict resolution, as sex-biased expression alone does not necessarily imply the past existence of sexual antagonism [47]. All three scenarios in Fig. 1a are compatible with intralocus sexual conflict, and these patterns apply to approximately 50% of contigs with female-biased expression (53.7% for autosomal contigs and 51.0% for sex-linked ones), and approximately 60% of contigs with male-biased expression (60.4% for autosomal and 64.2% for sex-linked contigs) (Supplementary Fig. 6). The remaining genes with male or female-biased expression may have evolved sex-biased expression under other selective forces acting during the evolution of separate sexes, for example to compensate for negative pleiotropic fitness effects of the sterility mutations involved, and of primary expression changes, or because upstream regulatory elements causing

sex-biased expression may affect other genes where the change is maladaptive.

We conclude that the sex chromosomes, as in animals, contribute to sexual dimorphism in *S. latifolia*, as genes with sex-biased expression are over-represented on these chromosomes. However, in contrast to animals with evolutionarily much older sex chromosomes [6,7,40,48], our analysis of the contribution of X and Y-linked alleles to sex-biased gene expression in this plant detected no accumulation of genes with female-biased expression on the X chromosome, or male-biased expression on the Y. Instead, we find evidence for opposing selective forces acting on the *S. latifolia* X and Y chromosome, leading to tertiary expression differences between X- and Y-linked alleles, a subtle form of feminisation of the X chromosome, and masculinisation of the Y. These expression changes may represent a hitherto unknown early stage of sex chromosome evolution that precedes the accumulation of genes with expression biased to one sex or the other.

Our study demonstrates considerable expression changes in this recently evolved dioecious species and illustrates the value of studying closely related species, only one of which has evolved separate sexes and sex chromosomes. Including a close relative with hermaphrodite flowers allowed us to infer that sex-biased gene expression in dioecious *S. latifolia* has evolved primarily through secondary expression changes of many genes in females. Our results support the long-standing hypothesis that the evolution of sex-biased gene expression reduces the detrimental effects of sexually antagonistic alleles present in the ancestral

hermaphrodite that became fixed at autosomal, and particularly at sex-linked, loci in the dioecious descendant. This resolution often reduces expression in the disadvantaged sex, but sometimes increases expression in the sex in which a derived allele was favoured in the dioecious descendant. Our comparative approach can be used to compare expression changes in genes that became fully sex linked at different times during the evolution of a sex chromosome system, and can be applied to studying other plant and animal lineages in which separate sexes have evolved recently.

Methods

Transcriptome sequencing, assembly and identification of sex-linked genes

Multiple females and males of *S. latifolia* and hermaphrodite individuals of *S. vulgaris* were used in this study (Supplementary Table 1). High quality RNA was extracted from small flower buds at developmental stages B1-B2 [49] and from fully developed rosette leaves. Individually tagged RNA-seq libraries were sequenced on Illumina HiSeq2000 machines using 100bp paired-end reads.

Illumina short reads and Roche 454 Genome Sequencer FLX reads derived from multiple tissues of *S. latifolia* were combined to assemble *de novo* a reference transcriptome encompassing 46,178 contigs (for details see Supplementary Methods). We used SEX-DETector [27] to classify

425 contigs as autosomal, sex-linked, X-hemizygous or undefined based on
426 SNPs segregating in one *S. latifolia* population.

427 **Analyses of sex-biased expression**

428 For gene expression analysis, RNA-seq reads derived from flower buds
429 and rosette leaves of *S. latifolia* and *S. vulgaris* were individually mapped
430 to the reference transcriptome. Numbers of mapped reads were extracted
431 per contig and sample and significantly differentially expressed contigs
432 between female and male *S. latifolia* individuals were identified in both
433 flower buds and rosette leaves.

434 Apparent sex-biased gene expression in flower buds of female and male
435 plants may arise trivially, when genes with sex-limited expression (i.e.
436 genes that are expressed in one sex only) are not expressed in the sex
437 that does not form the corresponding tissue (for example, apparent male-
438 biased gene expression may occur in *S. latifolia* for anther-specific genes,
439 simply because no anthers are formed in female flowers and the
440 corresponding genes are not expressed), or when genes are expressed
441 at similar levels in both female and male organs, and thus have reduced
442 expression when the organs are not developed in one sex. These contigs
443 representing primary expression changes (Fig. 1b) were excluded from
444 further analyses. They encompassed 839 contigs that were expressed
445 exclusively in males, and 64 contigs expressed exclusively in females.

446 To assess differences in the prevalence of contigs with sex-biased versus
447 unbiased expression, and female-biased versus male-biased expression
448 between autosomal and sex-linked contigs, we used Fisher's exact tests.

Expression divergence between *S. vulgaris* and *S. latifolia.*

To test whether directional selection has affected expression levels, we used the ΔX approach [35,36]. ΔX was calculated as $\Delta X = \text{mean}(X_{\text{SL females or males}})\text{-mean}(X_{SV})/\text{sd}(X_{\text{SL females or males}})$.

Expression differences between *S. latifolia* females or males and *S. vulgaris* hermaphrodites were divided by the standard deviation for all contigs, estimated separately for the two sexes in *S. latifolia*. For categories I and II of Fig. 1a, we computed the percentages of contigs displaying outlier expression divergence values between the two species (defined as $\Delta X \geq 75$ percentile across all contigs).

Allelic expression estimates of sex-linked contigs

Allelic contribution of the X and Y chromosomes to gene expression of sex-linked genes were calculated using sex linked SNPs and were compared to the autosomal ancestral stage in *S. vulgaris* (Supplementary Methods).

Data availability

RNA-seq data and the reference transcriptome have been deposited in the European Nucleotide Archive (ENA) under accession number PRJEB14171. Further data that support the findings of this study are available from the corresponding author upon reasonable request.

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619 Author contributions

620 Designed the experiments and formulated predictions: N.Z., A.W.,
621 G.A.B.M., D.C.; Performed the experiments: N.Z.; Analysed the data N.Z.,
622 R.T., A.M.; Wrote the manuscript: N.Z., A.W., D.C., G.A.B.M., R.T., A.M.

623 Competing interests

624 The authors declare no competing financial interests.

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627

Figures

Fig. 1

Hypothetical scenarios for the evolution of sex-biased gene expression. **a**, Evolution of female-biased expression from a hermaphroditic ancestral state. I: expression increased in females, II: expression decreased in males, III: expression increased in females and decreased in males. **b**, Evolutionary changes in gene expression associated with the transition from hermaphroditism to dioecy and the evolution of non-recombining sex chromosomes. Primary mutations lead to gynodioecy (or androdioecy) and subsequently to dioecy [50]. Secondary expression changes lead to sex-biased gene expression. Tertiary expression changes on sex chromosomes cause feminisation or masculinisation of X and Y (or Z and W) chromosomes.

642 **Fig. 2**

643 **Sexual dimorphism and sex-biased gene expression in *S. latifolia*. a**,
644 Sexual dimorphism in female and male flowers. **b**, Proportions of contigs
645 with female-biased (red), male-biased (blue) and unbiased (white)
646 expression for 12,708 contigs with inferred autosomal inheritance and
647 936 fully sex-linked contigs in flower buds and rosette leaves.

648

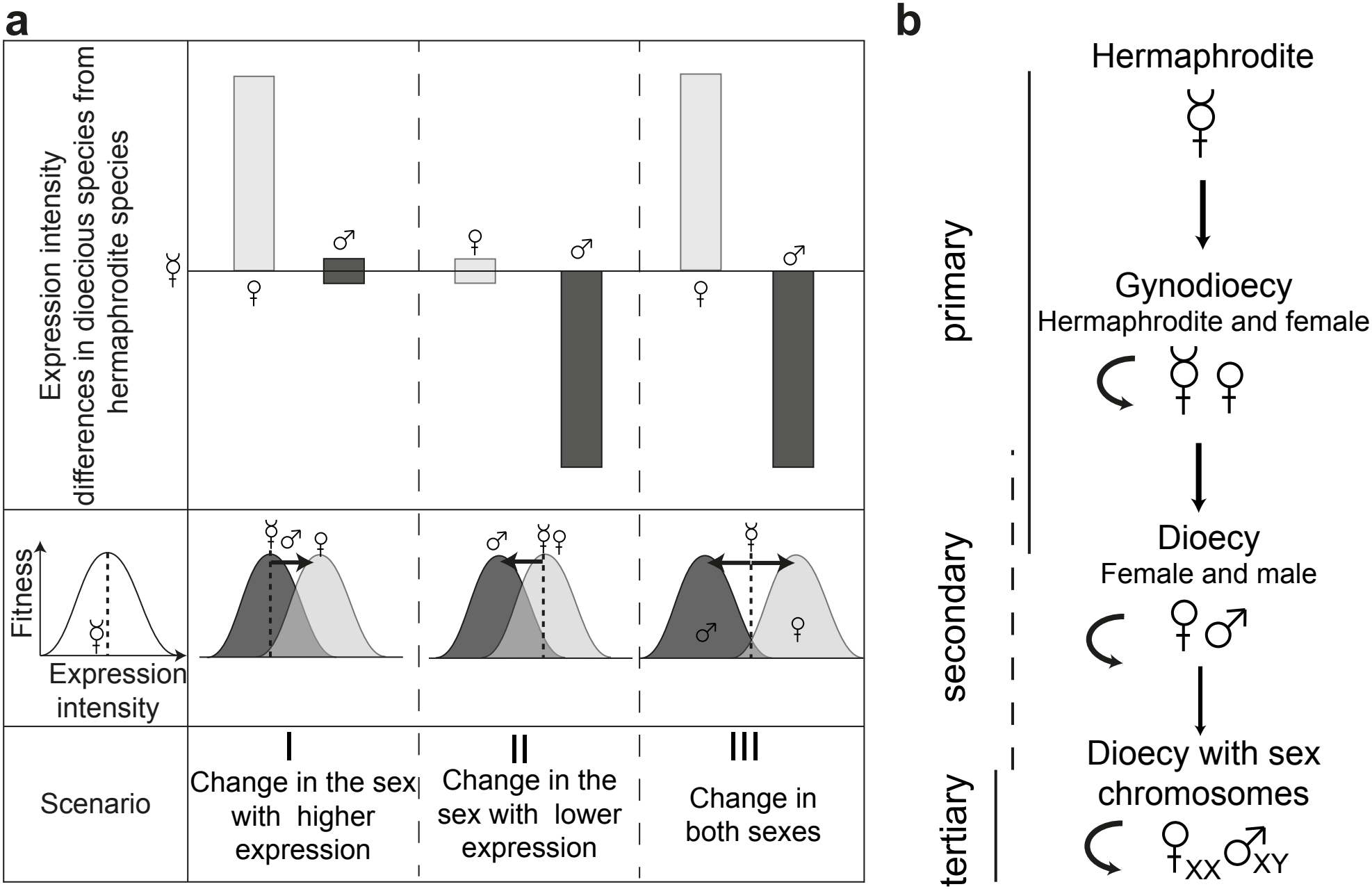
Fig. 3

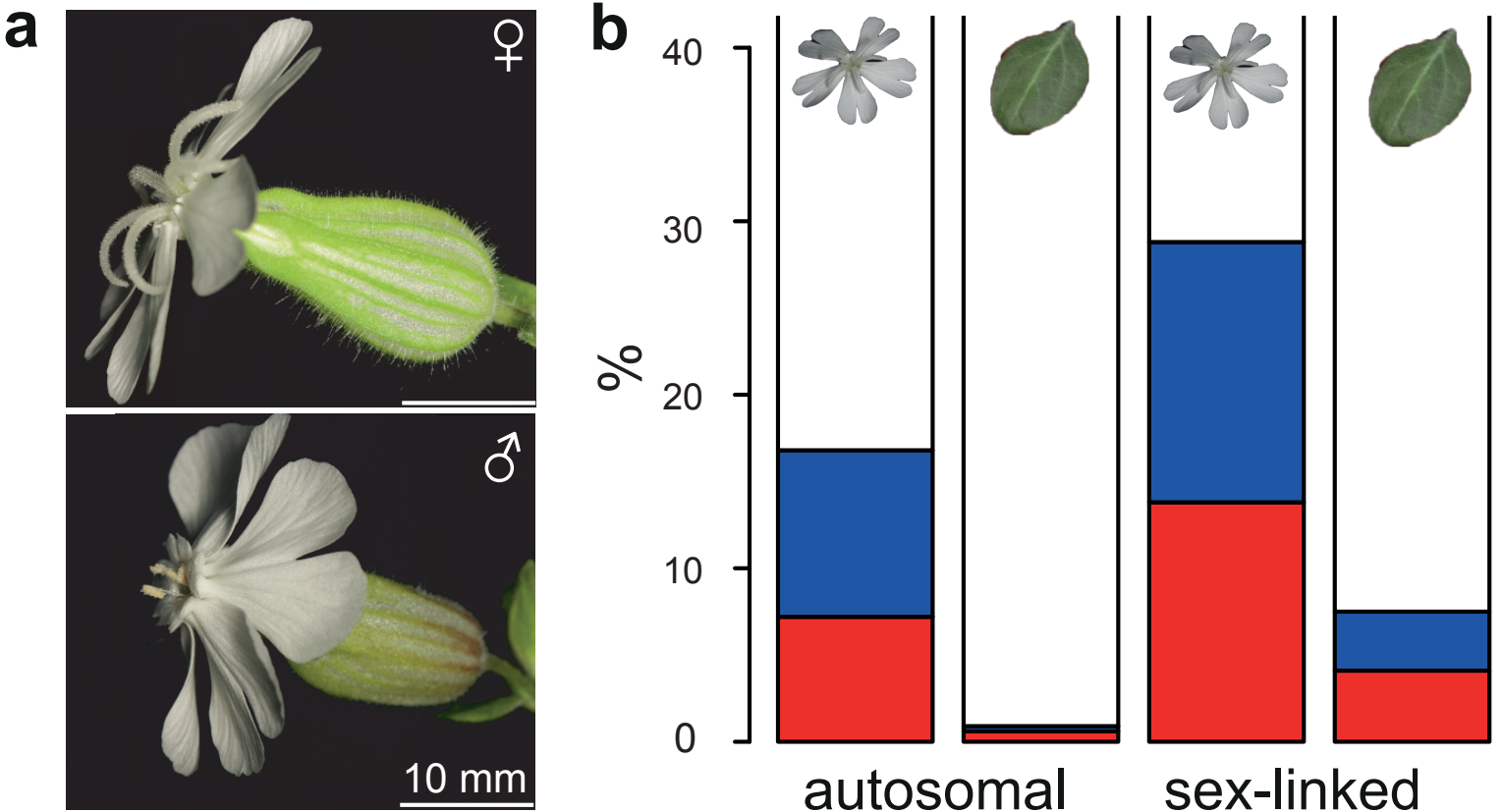
Expression changes in genes with sex-biased expression in *S. latifolia*. a-b, Expression differences (median with 95% confidence intervals) in (**a**) autosomal and (**b**), sex-linked contigs between *S. latifolia* females and males relative to *S. vulgaris* hermaphrodites for contigs with female-biased (red), male-biased (blue), and unbiased (white) expression in flower buds. Positive values correspond to genes with higher expression than in *S. vulgaris* hermaphrodites.

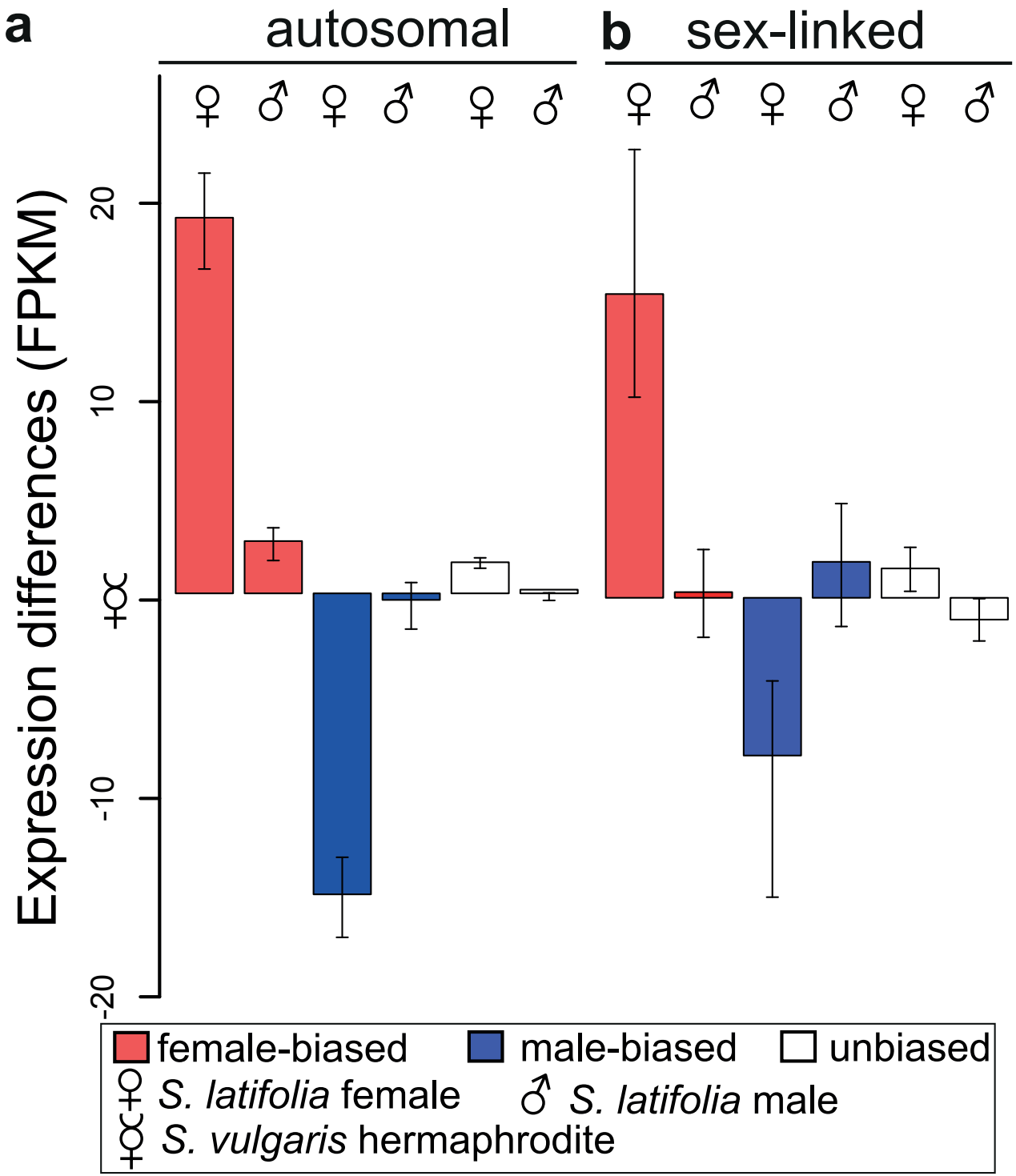
Fig. 4

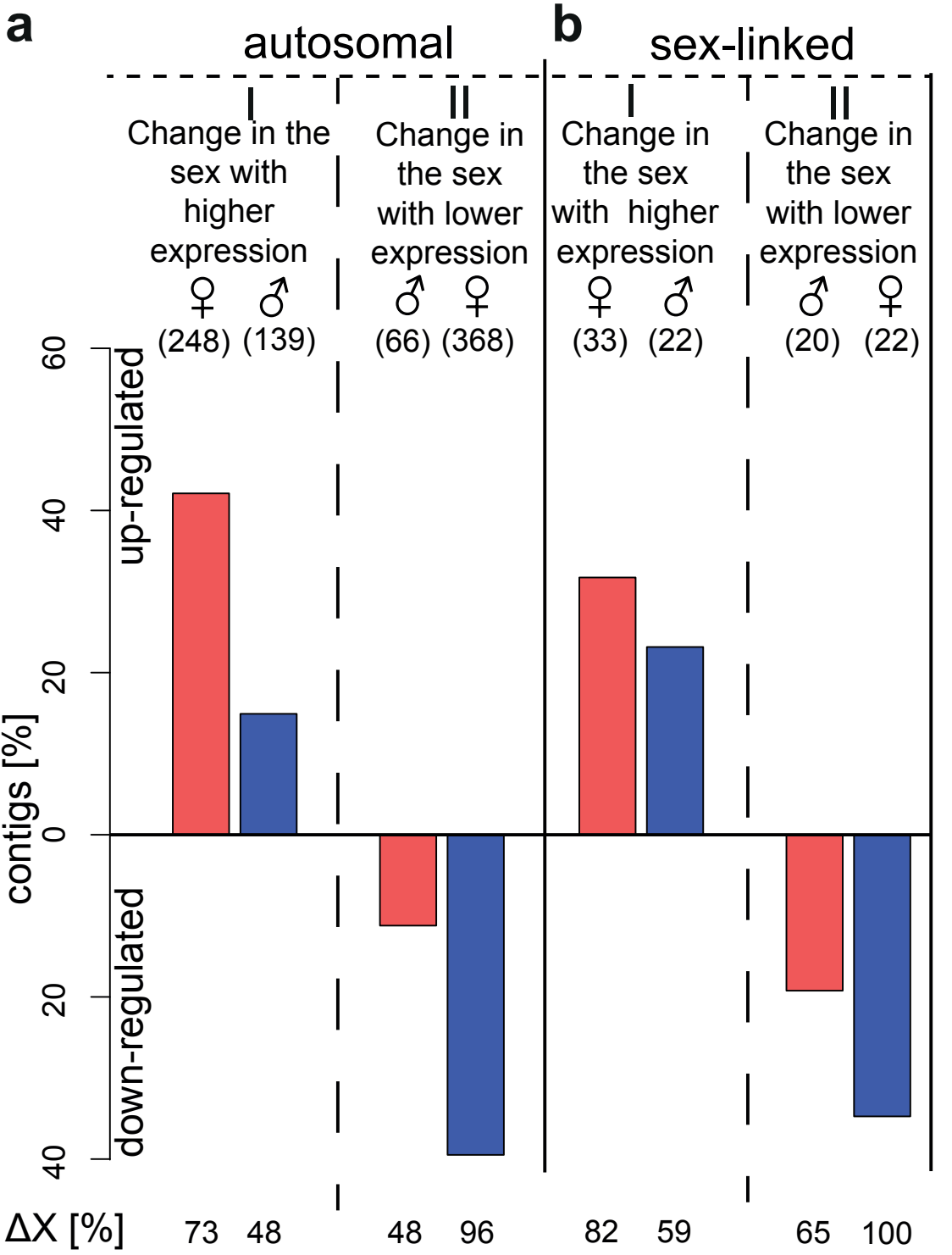
Evolutionary changes leading to sex-biased gene expression in *S. latifolia*. a-b, Autosomal (**a**) and sex-linked (**b**) contigs with elevated expression in the sex with higher expression (scenario I in Fig. 1a) and reduced expression in the sex with lower expression (scenario II of Fig. 1a) relative to *S. vulgaris* hermaphrodites for female- (red) and male-biased (blue) contigs. ΔX values indicate percentages of contigs that are outliers for expression divergence. **c**, Contigs with significant expression changes in opposite directions in the two sexes (scenario III in Fig. 1a). Numbers in parentheses correspond to numbers of contigs in each category.

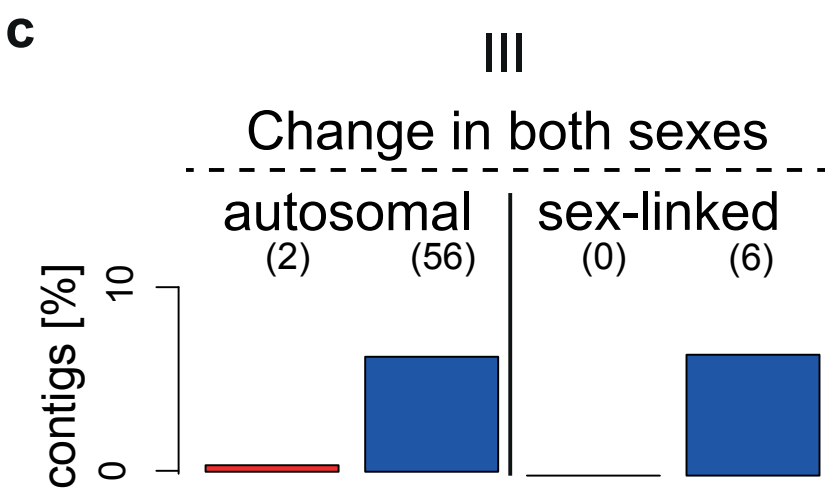
669 **Fig. 5: Tertiary expression changes on *S. latifolia* sex chromosomes.**
670 **a**, X female/male expression ratios in flower buds for contigs with female-
671 biased (red), male-biased (blue), and unbiased expression (white). This
672 ratio is significantly larger for contigs with female- than male-biased or
673 unbiased expression (Wilcoxon-test, $p \leq 0.0001$). **b**, Expression in males
674 as the ratios of Y expression to that in the cosexual ancestor for the same
675 three expression bias categories (colours as in a). Compared with contigs
676 with unbiased expression, ratios are significantly larger for male-biased
677 and smaller for female-biased contigs (Wilcoxon-test, $p \leq 0.01$ and $p \leq$
678 0.0001, respectively).


a
Expression intensity differences in dioecious species from hermaphrodite species
Fitness
Expression intensity
Scenario
I
Change in the sex with higher expression
II
Change in the sex with lower expression
III
Change in both sexes
b
Hermaphrodite
Gynodioecy
Hermaphrodite and female
Dioecy
Female and male
Dioecy with sex chromosomes
primary
secondary
tertiary


a
♀
♂
10 mm
b
40
30
20
10
0
%
autosomal
sex-linked


a
autosomal
b
sex-linked
♀
♂
Expression differences (FPKM)
20
10
-10
-20
female-biased
male-biased
unbiased
S. latifolia female
S. latifolia male
S. vulgaris hermaphrodite


a
autosomal
b
sex-linked
I
II
I
II
Change in the sex with higher expression
Change in the sex with lower expression
Change in the sex with higher expression
Change in the sex with lower expression
♀ ♂
(248) (139)
♂ ♀
(66) (368)
♀ ♂
(33) (22)
♂ ♀
(20) (22)
up-regulated
down-regulated
contigs [%]
60
40
20
0
20
40
ΔX [%]
73 48
48 96
82 59
65 100


c
III
Change in both sexes
autosomal
sex-linked
(2)
(56)
(0)
(6)
contigs [%]
10
0

a
b
$\log_2(0.5 \cdot XX_{females}/X_{males})$
$\log_2(Y/0.5 \cdot AA_{SV})$
10
5
0
-5
-10
***
***
**
***